201-15023

Diglyme

CAS Number 111-96-6

$$Me\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}Me$$

U.S. EPA HPV Challenge Program Submission

29 December 2003

Submitted by:

Ferro Corporation
1000 Lakeside Avenue
PO Box 147000
Cleveland, OH 44114

Prepared by:
Toxicology and Regulatory Affairs
1201 Anise Court
Freeburg, IL 62243
618-539-5280

Table of Contents

Executive Overview

Diglyme, CAS no. 111-96-6, is an inert ethereal organic solvent that is not known to occur in nature. It is synthesized from ethylene oxide and methanol with production of over a million pounds per annum in the United States. It is a clear, water-like liquid with a mild "aromatic" odor. Diglyme is of relatively low volatility for an organic solvent with a vapor pressure of 3.49 hPa @ 25˚C. It has a freezing point of -68°C and a boiling point of 162°C. and is miscible with water and most organic solvents. Because of its excellent solvating properties, its most extensive use is as a solvent for applications where an inert solvent is required or where its superior solvent properties are an important consideration.

In the environment, based on physicochemical properties and experimental data, diglyme will not bioaccumulate (log $K_{o/w}$ = - 0.36) and will distribute primarily to water and secondarily to soil where it will be subject to volatilization and slow biodegradation under conditions favorable to bacteria. It is stable to hydrolysis but expected to react rapidly with atmospheric hydroxyl radicals with a half-life of about 7 hours. Diglyme is relatively nontoxic to aquatic species, with an acute LC_{50} for freshwater fish greater than 2000 mg/L, an EC_{50} for daphnia of greater than 1000 mg/L and an EC_{10} for green algae greater than 1000 mg/L. Diglyme is considered inherently biodegradable in the environment but is suffiently resistant to biodegradation that a significant portion of it may will be released unchanged from a typical waste water plant. The primary fate of diglyme in the environment is volatilization to the atmosphere were it is rapidly photo-degraded and aerobic biodegradation in water and soil with a half-life estimated as several weeks. Even with the limited biodegradation rate it presents little environmental hazard due to low toxicity to aquatic organisms.

The acute oral LD_{50} of diglyme is very high with a value of about 5000 mg/kg being reported for rat gavage studies. Exposure of rats to saturated vapor for 7 hours did not produce any significant adverse effects that were macroscopically visible at necropsy. The dermal LD_{50} in experimental animals in unknown but based on human skin absorption studies and a "read across" approach using analogs is expected to be greater than 2000 mg/kg in the rabbit. As with most glycol ethers, dermal absorption is viewed as a potentially significant route of exposure.

Repeated-dose studies by the oral or inhalation route; demonstrate that the male reproductive organs followed by the bone marrow are important target organs for high-level diglyme exposures. Although low-level exposures are well tolerated, evidence in experimental animals indicates the potential for serious adverse effects in with overexposure. Metabolic studies in animals indicate that 2-methoxyacetic acid is a minor but variable metabolic product of diglyme. As 2-methoxyacetic acid is considered to interfere with cellular proliferation, tissues with rapidly proliferation are both predicted to be and are in fact target organs in experimental animals. These tissues include the testes (sperm production) the bone marrow (blood cell production) and, in pregnant experimental animals, the developing conceptus.

Adequate *in vitro* tests of genetic toxicity for diglyme are available. Multiple *Salmonella typhimurium* reverse mutation assays show lack of mutagenic activity in the presence or absence of metabolic activation and *in vitro* DNA damage and chromosome aberration studies have produce negative results. A study investigating the in

vivo genotoxicity of diglyme after inhalation exposure indicated a lack of genotoxic activity as evidenced by no increase in bone-marrow cell chromosome aberrations after exposures to levels of diglyme that cause testicular damage.

Developmental toxicity has been investigated in rats using inhalation as the route of exposure, and investigated in mice and rabbits using oral administration. The rat and mouse study provided acceptable evidence o specific developmental toxicity from exposure of animals to diglyme. The rabbit study is less clear since the maternal NOAEL is stated as being lower than the developmental NOAEL; nevertheless, the effect on concepti are of sufficient magnitude to indicate specific developmental toxicity in the face of adverse maternal effects

The combination of developmental toxicity findings in treated pregnant animals and testicular toxicity in repeated dose studies indicates the potential of finding adverse reproductive effects. There is additional evidence that treated and affected males lose actual reproductive capacity. This evidence comes from a study designed to investigate if diglyme produced a dominant lethal effect. In this study, groups of male rats were exposed to diglyme vapor for five consecutive days and then were mated weekly without further treatment. High-dose treated males were unable to produce many offspring from weekly matings between week 5 and 9 after exposure. This is indicative of a male-mediated adverse reproductive effect of diglyme in experimental animals

It is concluded that the available information adequately fills all the data elements of the HPV. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, they provide adequate to meet all the screening data recommendations. Conduct of additional screening studies would not add significantly to our understanding of this material's hazard. Although studies beyond the screening set could provide valuable information relating to human hazard and risk assessment, such studies are beyond the scope of the current evaluation and no new HPV screening studies are recommended.

Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 111-96-6 Diglyme	Information Available?	OECD Study ?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	Y	N	Y	N	
Boiling Point	Y	N	N	Y	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Partition Coefficient	Y	N	N	Y	N	Y	N	
Water Solubility	Y	N	N	Y	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	N	Y	Y	N	
Water Stability	Y	N	N	Y	N	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	Y	N	Y	N	Y	N	
Ecotoxicity								
Acute Fish	Y	N	Y	Y	N	Y	N	
Acute Invertebrate	Y	Y	Y	Y	N	Y	N	
Acute Algae	Y	Y	Y	Y	N	Y	N	
Toxicity								
Acute	Y	N	Y	Y	N	Y	N	
Repeated Dose	Y	N	Y	Y	N	Y	N	
Genetic Toxicology *"in vitro"*	Y	N	Y	Y	N	Y	N	
Genetic Toxicology "*in vivo*"	Y	N	N	Y	N	Y	N	
Reproductive	Y	N	N	Y	N	Y	N	
Developmental	Y	Y	Y	Y	N	Y	N	

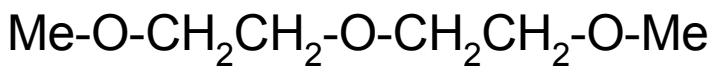
Introduction

Diglyme, CAS no 111-96-6, is a linear aliphatic diether that is a clear liquid at room temperature with what is described as a mild odor (1). It is miscible with water and organic solvents (2) and, because of its chemical inertness and excellent solvent properties, finds use as a specialty solvent for a wide variety array of applications. Diglyme is used as a reaction solvent for Grignard-reactions, reduction-reactions, alkylation-reactions, and organo metallic reactions in general. It finds application in reactions involving alkali metals such as lithium, sodium and potassium and can dissolve Na/K alloy, but potassium is only sparingly soluble. It dissolves vinyl chloride copolymers, polymethacrylate, polystyrene, polychloroprene and cellulose acetate (3). Other applications include in the coating industry and in photolithography for manufacture of semiconductor chips.

Diglyme is most commonly produced by reacting ethylene oxide with methanol in the presence of either acidic or basic catalysts although it can be made from diethylene glycol and dimethyl sulfate (HSDB). Occupational exposure during the manufacturing process is minimal as the production is in a closed system that is designed to provide adequate protection from the reactant ethylene oxide, which is a regulated chemical. Significant worker exposure is only possible during sampling, drumming and equipment maintenance.

No ACGIH TLV or other regulatory limit was located for diglyme. NIOSH, however, recommends reducing exposure for all glycol ethers to the lowest feasible concentration and preventing contact with the skin. (4).

Diglyme's structure is shown below:



Diglyme

Diglyme is also known as (5):

- Ethane, 1,1'-oxybis[2-methoxy- (9CI)
- Ether, bis(2-methoxyethyl) (8CI)
- Bis(2-methoxyethyl) ether
- Diethylene glycol dimethyl ether
- Diethyl Glycol Dimethyl Ether
- Diglycol methyl ether
- Diglyme
- 1,2-Dimethoxyethane
- Di(2-Methoxyethyl) ether

- Dimethyl carbitol
- Ethanol, 2,2'-oxybis-, dimethyl ether
- Ethylene glycol dimethyl ether
- Glyme-2
- 2-(2-Methoxyethoxy)-1-methoxyethane
- (2-Methoxyethyl) ether
- Methyldiglyme
- 1,1'-Oxybis(2-methoxyethane)
- Poly-Solv
- Poly-Solv D2M
- 2,5,8-Trioxanonane

Several physicochemical, fate and toxicity studies have been conducted on diglyme. These studies are briefly reviewed in this testing rationale document, which also describes how these studies meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries or given as shorter summaries using the IUCLID format. The available data set satisfactorily fulfills the data requirements for the EPA HPV Program. The majority of data elements are filled by high-reliability studies on diglyme. Where direct data are not available or data are sparse, surrogates or estimation methods are used to fill the data element. The U.S. EPA and other regulatory authorities encourage this approach, where scientifically defensible, to avoid unnecessary testing and animal usage.

Physicochemical Data

Physicochemical data for Diglyme are available from the literature.

Table 1: Physicochemical Properties of Diglyme

Melting Point	-68° C (2)
Boiling Point	162° C @ 1010 hPa (2)
Vapor Pressure	3.49 hPa @ 25˚ C (6) (2.96mm)
Partition Coefficient	Log $K_{o/w}$ = -0.36 (7)
Water Solubility	Miscible (2)

These properties indicate that at ambient temperatures, Diglyme is a slightly volatile liquid with unlimited water solubility. The value of the partition coefficient suggests that Diglyme will partition preferentially into water;

therefore, only on the basis of the octanol-water partition coefficient, Diglyme is considered to have little potential for bioaccumulation.

Molecular Formula	C6-H14-O3
Molecular Weight	134.17
SMILES Code	COCCOCCOC

Recommendation: No additional physicochemical studies are recommended. The available data fill the HPV required data elements.

Environmental Fate and Pathways

Multiple investigations have been conducted on the biodegradation of diglyme and the data consistently indicate that diglyme can be aerobically biodegraded but is resistant to biodegradation in the usual screening tests. Tessier reported, in 1983, that highly oxygenated compounds, including diglyme, are difficult to degrade and when biodegradation does occur, it occurs slowly (8). Grant chemical (now Ferro) supported a study of the biodegradation of 1,4-dioxane and diglyme by adapted microorganisms. Results of this study, published by Roy et al (9) indicted that there was a population of bacteria in the waste treatment plant that could biodegrade diglyme but they apparently prefer other organic substrates to diglyme. Using a seed of dioxane/diglyme-adapted bacteria, pure diglyme, after a seven-day lag phase, was only partially (36%) degraded in 25 days of incubation. Diglyme in mixtures of dioxane and diglyme that were inoculated with these adapted bacterial was degraded by 85% in the presence of dioxane. The diglyme degrading bacterial population was also found to be inhibited by salt concentrations of one percent and greater. It was also postulated that dioxane is metabolized by these bacteria to a toxic product that inhibited further biodegradation when dioxane concentrations were suffiently high. Overall, these studies show that diglyme is biodegradable but is a poor substrate for even adapted bacteria. They also indicate the importance of a mixed population of bacteria and substrates to achieve optimal biodegradation.

Cowan and Kwon (10) extended the idea of adapting bacteria to mixed cultures of glycol ethers and other difficult to biodegrade ethers. They started with a bacterial culture from a petroleum refinery wastewater treatment plant and established these bacteria in a 5-liter Submerged Attached Growth Air Lift reactor (where the bacteria adhere to a fibrous support media and have an essentially infinite residence time). After 34 weeks of operation and optimization, the reactor effectively removed five of ten glycol ethers in the mixture, in the best case the diglyme removal by the adapted bacteria about 40%. This removal is better than that for four other glycol ethers that were only removed between 10 and 25% under optimized conditions this information allows a rough rank ordering of glycol ether's resistance to biodegradation which indicates that diglyme should be considered difficult to biodegrade, but not recalcitrant.

This classification is supported by two OECD guideline studies, one conducted by Hoechst AG and reported by IPCS (11) and the other by the European Chemicals Bureau (12). In the first test, a closed bottle test according to

OECD 301D, only 0.1% degradation (measured as oxygen uptake) was recorded in the first days of exposure and no 28-day degradation is reported (13). The second study was a modified Zahn-Wellens test in which the results are reported as 31% biodegradation after 28 days in the ECB IUDLID-2000 document and as 42% after 28 days by IPCS in the 2002 CICAD document (14).

Grossmann (15) recently described studies on chemically assisted decomposition of diglyme prior to introduction in a waste treatment plant that effectively utilized hydrogen peroxide and ozone based advanced oxidation processes to allow mineralization of diglyme. Fenton, photo-assisted Fenton and UV/H_2O_2 oxidation processes all showed acceptable TOC removals.

Photodegradation of chemicals can occur by two major mechanisms; direct, where the molecule has a chromophore that absorbs light in the range of wavelengths that impinge on the earth's surface; and indirect, where the chemical reacts with an atmospherically generated sensitizer such as hydroxyl radical or ozone. As diglyme has no chromophore that absorbs light in the appropriate range, direct photolysis is considered unimportant as a mechanism of photolysis. Indirect photolysis by reaction with atmospheric hydroxyl radical is anticipated based on diglyme's chemical structure.

Indirect photolysis was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced an estimated rate constant of 29.7 E-12 cm^3/molecule-sec (see accompanying robust summary in Appendix for full details); however, the SRC database contained an experimental value recorded by Dagaut and reported by Atkinson of 17.5 E-12 cm^3/molecule-sec (16). This experimental value is about half the value calculated by AOPWIN based on the chemical structure but both still predict a relatively short atmospheric half-life. The model predicted half-life, assuming a concentration of 1,500,000 hydroxyl radicals per cubic centimeter, is 4.3 hours. Using the experimental rate constant, the estimated atmospheric half-life is 7.33 hours. Given the preference for experimental data over calculated, and that the half-life derived from the experimental data is a more conservative number, the 7.3 hour atmospheric half-life for diglyme is accepted and has been applied in the EQC Level III modeling of environmental distribution.

Although water stability has not been quantitatively determined for diglyme in an OECD 111 guideline study, the National Toxicology Program has conducted a dosing vehicle test of aqueous diglyme solutions and concluded that it is stable in aqueous solution for a period of 21 days (17). In addition, water stability studies are considered unnecessary for compounds containing only non-hydrolysable groups. There is no evidence available in the literature that diglyme is unstable in water and the structure is that of a simple aliphatic ether, which is a class of molecule considered to be water unreactive at environmental pH values. The half-life in water is thus estimated as

greater than one year. This assessment is confirmed by the review of Harris, who notes specifically that ethers as a class are non-hydrolysable (18). Additional evidence attesting to its stability under basic conditions comes from its application as a preferred solvent for chemical reactions that occur under extremely basic conditions (19).

Theoretical Distribution (Fugacity) of diglyme in the environment was estimated using the MacKay EQC level III model with standard defaults in EPIWIN v 3.05 but using the measured vapor pressure of 2.96 mm Hg, the measured log $K_{o/w}$ of -0.36, measured melting point, experimentally based photolysis estimate and data derived estimates of biodegradation (20). The results for environmental distribution using a model calculated $K_{o/c}$ (adsorption coefficient based on organic carbon content) of 0.179 and equal initial distribution to air, water and soil are:

- Air 0.505 %
- Water 60.4 %
- Soil 38.9 %
- Sediment 0.113 %

Recommendation: No additional fate studies are recommended. The available data fill the HPV required elements.

Ecotoxicity

Acute fish, invertebrate and alga studies have all been conducted for diglyme. Results of these studies and the ECOSAR predicted values using the neutral organics model are given in the table below.

Table 2: Comparative Aquatic Toxicity of Diglyme

	Reported Experimental Values	ECOSAR Prediction
Fish, 96 hour LC_{50} (DIN Guideline study)	> 2000 mg/L (21)	16,446 mg/L*
Daphnia, 48 hour EC_{50} (OECD Guideline study)	> 1000 mg/L (22)	14,971 mg/L*
Alga, 72 hour EC_{10} (OECD Guideline study)	> 1000 mg/L (23)	8,171 mg/L*

* Estimated using ECOSAR (24)

All of these studies have been conducted in accord with an appropriate guideline but the results are only available in the ECB IUCLID-2000 document (fish) or the IPCS CICAD document (daphnids and green algae) for diglyme. Robust summaries have been prepared giving what information is available to the public about these studies. Without the original reports to review, many details cannot be checked; however, the extensive international review by experts in ecotoxicity that the CICAD document receives prior to publication assures that there is established consensus surrounding these data. Other factors that were taken into considerations in recommending these studies be accepted for the EPA HPV program are that 1) with a highly water soluble, low volatility and stable material such as this, details of study conduct and verification of test substance are of less importance. 2.) Numerous materials of related structures have been evaluates in acute aquatic toxicity screening tests and have shown minimal toxicity to aquatic species. 3.) Quantitative SAR modeling (ECOSAR) predicted values for adverse effect levels from diglyme exposure are exceeding high and this QSAR model is typically accurate in predicting toxicity values for non-reactive and difficult to activate materials such as diglyme. Thus, although it is recommended that these data be accepted as fulfilling the requirements of the U.S. EPA HPV program, they are assigned reliability scores of 2 due to only being available in the secondary literature.

Recommendation: The fish, invertebrate and algal tests are adequate for the purposes of the HPV program screening evaluation. No additional aquatic screening studies are recommended.

Absorption, Distribution, Metabolism and Elimination

Absorption:

Multiple studies on the metabolism of diglyme in experimental animals demonstrate that diglyme is rapidly and completely absorbed from the gastrointestinal tract of the rat (28, 29) and the mouse (25). Toxicity following inhalation exposure confirms that diglyme is absorbed from the lungs, especially in the studies employing nose-only exposure (vide post). Dermal absorption has been shown in vitro using human skin and actually measuring the rate of movement across human skin (38). These results suggest that diglyme will be absorbed through the skin in vivo. The physicochemical properties of diglyme ($K_{o/w}$, neutral charge, molecular mass and excellent solvating properties) are also consistent with a material that will be absorbed by all routes of exposure.

Metabolism:

The major pathways for metabolism of diglyme have been identified in the rat and an understanding of this metabolism is helpful in understanding the toxicity of diglyme to experimental animals and man. Figure 1 indicates there are two initial metabolic oxidations that occur, with both implicated to involve Cytochrome P-450. The first, labeled as "Path A" is an oxidative dealkylation of an interior ether bond to formally give two molecules of 2-methoxyethanol; which has been extensively investigated with regard to its metabolism and toxicity. 2-Methoxyethanol is oxidatively converted, by way of the aldehyde, to 2-methoxyacetic acid (shown on the figure as bolded). 2-Methoxyacetic has been associated with testicular toxicity in male experimental animals and development of the conceptus in pregnant female animals In rats, most of the 2-methoxyacetic acid is excreted in the urine but some is conjugated with glycine to produce the amide N-methoxyacetyl glycine.

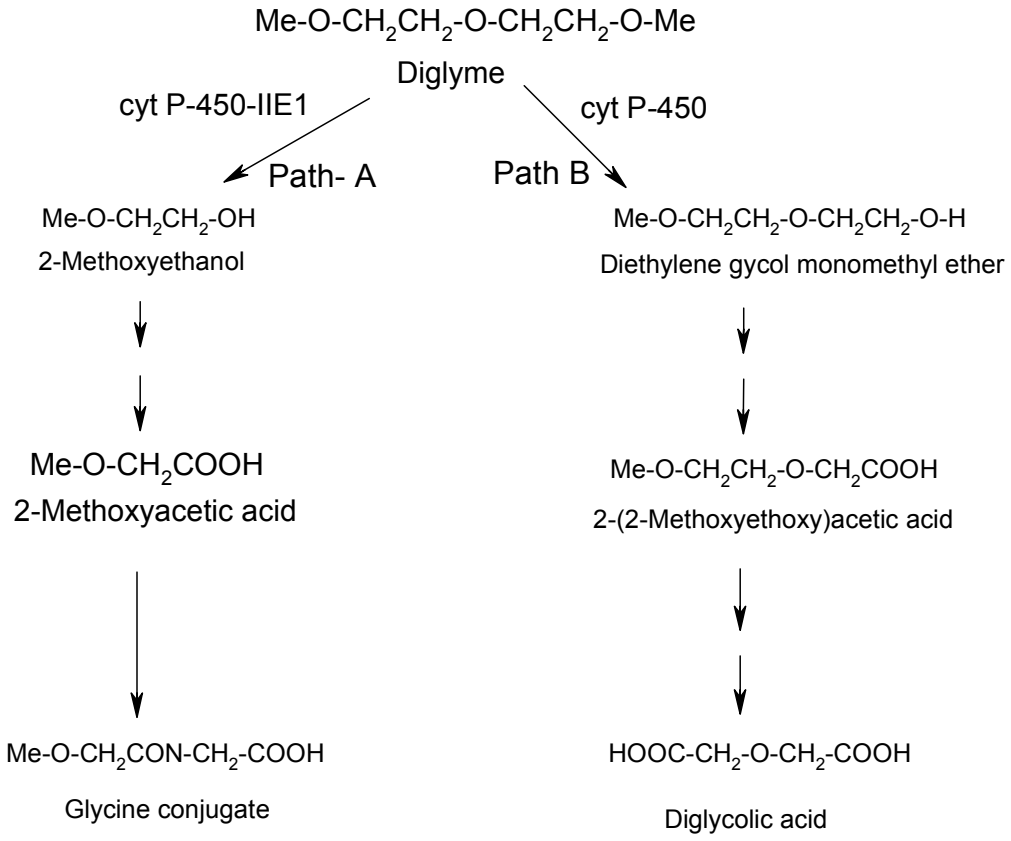


Figure 1: Metabolic Pathways for Diglyme in the Rat

Pathway B, involves oxidative demethylation of diglyme, by unspecified cytochrome p-450 isozymes to give 2-(2-methoxyethoxy)ethanol; which is oxidatively converted, by way of the corresponding aldehyde, to 2-(2-methoxyethoxy)acetic acid. Another oxidative demethylation on the other end of this molecule gives the alcohol 2-hydroxyethoxyacetic acid (not shown) that will be oxidized, through the corresponding aldehyde to the diglycolic acid and excreted.

Human and rat liver microsomal preparations have been shown to produce qualitatively and quantitively similar oxidative metabolic products suggesting that the human pathways for diglyme may be similar to those observed in experimental animals (26). In another report, it was stated that human-liver microsomes are even more efficient than rat-liver microsomes at cleaving diglyme into 2-methoxyethanol (27). Cytochrome P-450 induction by Phenobarbital, and to a lesser extent by diglyme itself, has been reported to increase the relative amount of diglyme that is metabolized to 2-methoxyethanol (28).

Studies in which rats were dosed with 2-(2-methoxyethoxy)ethanol or 2-(2-methoxyethoxy)acetic acid have been conducted and indicate that there is no "cross over" from the 2-(2-methoxyethoxy)ethanol pathway to produce the toxic metabolite 2-methoxyacetic acid (29). This indicates that which metabolic pathway diglyme will follow is primarily depended on the initial oxidative attach on the diglyme molecule. The initial pathway of attack is strongly influenced by the relative quantities of the various cytochrome P-450 isozymes that are present at any given time. Although no studies of human diglyme metabolism were found in the open literature, based on what is known about the metabolism of diglyme (vide supra) and what is known about the metabolism of glycol ethers in general (30), experimental results in animals are considered relevant to human hazard and risk assessment.

Health Effects

Acute Toxicity

Oral Exposure

Two acute oral studies on diglyme have been conducted on rats and one conducted on mice. These are shown in the following table.

Oral LD_{50} (mg/kg)	Species	Sex/strain	Comment	Reference
ALD = 7500	Rat	CD/male	Robust summary	31
4760	Rat	Wistar/female	From IUCLID-2000	32
2978	Mouse	CD-1/female	Published 1985	33

Table 3. Acute Oral Toxicity of Diglyme

The first study in the table is an "approximate lethal dose" (ALD) study conducted at eight dose levels using one animal per group. As the original report was available though NTIS, and the study has not been reviewed in any previous publication, a robust summary was prepared for this study and is included in the appendix. Due to the low statistical power of the ALD test, it cannot be determined if there is an actual difference in sensitivity between CD-1 male rats and Wistar females. All studies indicate a low order or acute oral toxicity for diglyme and no study identified a target organ.

Inhalation Exposure

The only specific acute inhalation study located for diglyme is an "inhalation risk test" using air "saturated" with diglyme, in which Wistar rats of each sex were exposed to diglyme vapor for 7 hours (34, 35). After exposure, animals were maintained for a 14-day observation period after which they were sacrificed and subjected to a gross necropsy. All animals survived exposure and a 14-day observation period. No macroscopic findings were observed at necropsy 14 days after the exposure. Clinical signs were restlessness, narrowing of palpebral fissures, and irregular breathing in rats. Based on the vapor pressure the concentration could have been as high as 3800 ppm (21 mg/L) but the actual nominal concentration was reported as > 11 grams/m^3, this is about 2000 ppm (see accompanying robust summary).

This result is supported by the results of repeated-dose studies reported by McGregor et al. (36), who exposed CD rats for 7 hours a day for 5 days at a measured diglyme concentration of 1000 ppm without mortality. It is also supported by the work of Valentine et al. (37) who exposed groups of 20 male and 10 female rats to measured concentrations of up to 1100 ppm diglyme, six hours a day five days a week for 10 exposures without mortality.

Dermal Exposure

No acute dermal studies were located for diglyme; however, there is a recent measurement of the ability of diglyme to penetrate human skin relative to six other glycol ethers published by Filon et al. (38). Eight other glycol ethers were evaluated by Dugard et al. using a similar method (39). An estimate of the likely skin penetrating ability of diglyme can be derived by examination of Table 4.

Structure (H_2's Excluded)	Common name	Rate of absorption (mg/cm^2-hr)		Rabbit Dermal LD_{50}	Rat Oral LD_{50}
		Dugard et al (38)	Filon et al (39)		
Me-O-CC-OH	2-Methoxyethanol	2.82		1300 (40)	3250 (40)
Me-O-CC-O-CC-O-Me	Diglyme		0.952		4760
Et-O-CC-O-Ac	2-Ethoxyethanol acetate	0.800		1818 (41)	2900-7500 (52)
Et-O-CC-OH	2-Ethoxyethanol	0.796	0.820	3311 (40)	2125-5487 (52)
Et-O-CC-O-Et	Ethylene glycol diethoxy ether	0.125	0.166		>4390 (42)

Table 4. Measured Human Skin Absorption Rates of Some Glycol Ethers.

Keeping in mind that the 95% confidence interval surrounding each determined rate of absorption is large, it can be surmised that diglyme probably penetrates skin with about the same facility as 2-ethoxyethanol and 2-ethoxanol acetate, both of which have dermal LD_{50} values for the rabbit equal to or lower than the rat oral LD_{50} values. Without attempting to correlate intrinsic toxicity with skin and intestinal absorption and toxicokinetic considerations, a simple read across approach suggests that the rabbit dermal LD_{50} for diglyme is in the range of 2000 to 4000 mg/kg. As the current practice for conducting dermal toxicity testing limits the top dose to 2000 mg/kg (43), it is unlikely that any significant information would be obtained by the actual conduct of a dermal study.

Recommendation: No additional acute toxicity studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, the weight of evidence shows that the oral and inhalation acute toxicity is very low. Sufficient information about the dermal penetration of diglyme absorption though human skin is available to assess the hazard of dermal toxicity. Conduct of additional acute-toxicity screening studies studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional acute toxicity studies be conducted.

Repeat Dose Toxicity

Oral Exposure

The only repeated dose oral study found I the literature that was not focused on reproductive function is a study in which four male JCL-ICR mice were treated with diglyme in the drinking-water for 25 days at a level of 2% (44). At the end of the exposure period the number of total white blood cells was more than twice that of controls. Although this finding is of possible interest the study design lacked the power to produce a statistically significant effect. Repeated-dose studies on potential effects of diglyme on male reproductive are found in the reproductive toxicity section. Inhalation is considered the more relevant route.

Inhalation Exposure

A modern inhalation study with analytically determined concentrations with recovery groups and a positive control compound (2-methoxyethanol) has been reported by Valentine et al. (45). In this study, groups of 20 male and 10 female rats were exposed to 0, 110, 370, or 1100 ppm diglyme, six hours a day five days a week for 10 exposures. Male rats were killed after 10 days of exposure and 14, 42, or 84 days post-exposure. Female rats were killed after the 10th exposure and 14 days latter. Urine analysis, hematological analyses, clinical chemistry and histopathology were performed. Changes in the hematopoietic system occurred in males and females involving

bone marrow, spleen, thymus, leukocytes, and erythrocytes. The NOAEL for female rats was 370 ppm. Males were more sensitive than females and the primary target organ for males was found to be the reproductive system. Stage-specific germ cell damage occurred at all concentrations and was concentration and time dependent Effects on the male reproductive system at a concentration of 110 ppm were relatively mild and can be considered a LOAFL. The effects produced by 300 ppm 2-methoxyethanol were more severe than produced by 370 ppm diglyme but not as severe as produced by 1100 ppm diglyme under the same conditions of exposure. It was concluded that diglyme is one-half to one-third as potent as 2-methoxyethanol under the same inhalation conditions.

As a NOAEL for testicular effects was not identified, a second study using the same design was performed with lower concentrations of diglyme (46). In this study, measured concentrations of 0, 3.1, 9.9, 30, or 98 ppm were used to expose rats by inhalation for 10 exposures followed by a 14-day recovery period. Mean body weights of rats exposed to 98 ppm were significantly lower than those of controls at the end of the exposure period. The weights of testes, seminal vesicles, prostate, and epididymides were similar to controls. Microscopic examination of the testes revealed minimal or mild testicular atrophy in the 100-ppm group. Findings at lower concentrations were not considered to be compound related. It was concluded that the target organ is the male reproductive system and the NOEL for male rats is 30 ppm under these conditions.

Recommendation: No additional repeated-dose studies are recommended. The available data fill the HPV required endpoint for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points: generally one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of this material, adequate tests have been conducted that cover both of these endpoints. Complementary studies have also been conducted and the overall weight of evidence indicates lack of genotoxic potential for diglyme.

Genetic Toxicology in vitro

Results from the *Salmonella typhimurium* reverse mutation assay have been negative. The NTP conducted and reported tests using microsomal activation system from two different species (47). Their findings indicate that neither rat liver or hamster-liver metabolic activating systems induces diglyme to have any mutagenic activity in this test system. The results from the NTP studies (48) have been summarized robustly and appear in the Appendix. Other Salmonella typhimurium reverse mutation assays have been conducted and reported to be negative. McGregor reported two of these (49) and the other is contained in two unpublished reports from Hoechst AG (50, 51).

Another DNA damage test was conducted using human cells (49). In this study, human embryonic intestinal fibroblasts were observed for unscheduled DNA synthesis after 3 hours of in vitro exposure to up to 19mg/ml diglyme and did not give a response indicative of diglyme causing any damage to human DNA

Genetic Toxicology in vivo

Information from un-confounded genotoxicity studies conducted *in vivo* is limited to a report in which bone marrow cells form groups of 10 CD rats of each sex exposed to 250 or 1000 ppm diglyme for seven hours a day for 1 or 5 days were examined for chromosome aberrations (49). An increase in chromosome aberrations was not observed in bone marrow cells of either sex at either dose. This study is considered and adequate test of the clastogenic potential of diglyme because it was conducted by a scientifically defensible method and because the high dose level was documented to produce severe toxicity to the testes of male rats exposed under the same conditions as described in the *Fertility* section of this document (and the robust summery covering fertility).

Other attempts at in vivo genotoxicity testing of diglyme were a recessive lethal test on *Drosophila melanogaster* that could not be evaluated because of an unusually high death rate in a control group and a dominant lethal test in rats (49). The rat study showed a reduced number of pregnancies and an increase in preimplantation losses but it could not be determined if this was due to a dominant lethal effect or to reduced fertility of the males. In light of the established adverse effects of diglyme on fertility, it was assumed that reduced fertility was the cause and not a dominant lethal effect. This is considered a reasonable assumption as diglyme showed no genotoxic activity in other assays and as the class of compounds known as glycol ethers is considered to have low potential for inducing genetic damage (52).

Recommendation: The SIDS requirement for genetic testing has been met as assays sensitive to both point mutation and to clastogenic effects have been conducted using acceptable protocols. No additional genotoxicity testing is recommended.

Reproductive Toxicity

The combination of the positive developmental toxicity studies in rats and mice with repeated dose studies (see repeated dose section and accompanying robust summaries) showing that the reproductive organs of male experimental animals is a specific target organ for diglyme, is sufficient information to fulfill the relevant requirement for reproductive toxicity information. In addition to this information, a dominant lethal test in rats is available that provides definitive information about the effect of diglyme on male reproductive function and, also

located in the literature, was a specific study of the effect of diglyme on the testes of Sprague-Dawley rats (*vide infra*).

Cheever et al. (53) investigated the effect of repeated orally administered diglyme on the pathology and lactate-dehydrogenase-X (LDH-X) levels of the testes of Sprague-Dawley rats. Daily administration of diglyme at 5.1 millimole/kg body weight diglyme to male Sprague-Dawley-rats resulted in reduced body weight gain and reduced testis weights. The testis weights revealed reductions after as few as ten doses while reduction in body weight gain only occurred after 18 consecutive daily doses. The testes, epididymides, and thymus glands of rats given 20 doses of diglyme demonstrated significant decreases in relative weights. Degenerative changes were noted on histopathological examination of the testes after 20 consecutive days of dosing and degenerative changes were noted in the germinal cells after as few as eight doses. Cessation of treatment resulted in some evidence of regeneration of spermatocytes beginning at 2 weeks after stopping treatment. Decreased LDH-X isoenzyme activity was noted in testis homogenates.

The dominant lethal test consisted of groups of 10 male adult CD rats exposed to 0, 250, or 1000 ppm diglyme for seven hours a day on five consecutive days, then serially mated at weekly intervals for 10 weeks to untreated virgin females (54, 49) (also see associated robust summary for this study). The presumed-pregnant female rats were killed and examined 17 days after they were first caged with the males. No effect on frequency of pregnancy was seen in the 250-ppm exposure group. Large reductions in pregnancy frequency, however, occurred in the 1000-ppm exposure group in weeks 4 through 9, especially in weeks 5 through 7 after exposure, when pregnancy frequencies were only about 10%. Most of the loss was attributable to preimplantation loss. Although transformation of the data allowed interpretation of the data that implied post-implantation loss in weeks 5 and 6, this conclusion was confounded by the know increase in post-implantations loss in dams where total implantation is low. In conclusion, the results of the test are equivocal regarding a dominant lethal effect and the authors concluded that the effect of diglyme "on male fertility and embryonic development are of much greater importance than genetic effects when setting tolerable limits"

The investigators focused on analysis of the data to investigate if there was a significant post-implantation loss as a test of genotoxicity, rather than looking on this primarily as a study of male fertility. Although it could not be ascertained if diglyme has any activity in causing a dominant lethal effect, the results of the study are very valuable in definitively demonstration a clear adverse effect of diglyme on functional male fertility. The design of the study also lends itself to indicating that the initial effects in the testes are primarily on the early stages of sperm development, probably the mitotic cells (spermatocytes) and not on the germinal epithelium or the haploid stages of the sperm, which were apparently unaffected in their ability to effectively cause pregnancy and viable fetuses. In addition, the study indicates that although the effects on sperm can be severe enough to restrict the induction of pregnancy, the effects are largely reversible in rats after 10 weeks of recovery (at least for males that are mated weekly).

Additional supporting information on the effect of diglyme on sperm comes from a "Sperm abnormality test in mice" reported by McGregor and co-workers (49) in which inhalation exposure of male mice to 1000 ppm

diglyme vapor for 7 hours a day for 4 consecutive days increased the incidence of sperm with "amorphous heads" from the control level of 2.2% to 20.9% upon examination 35 days after exposure. Other categories of sperm abnormalities were also significantly increased including "hook turned-up or elongated", "banana-shaped head" and "hair pin or tight coil tail". Exposure of mice to 250-ppm diglyme vapors for 7 hours/day for five days did not affect sperm morphology.

Recommendation: No additional reproductive testing is recommended, as the available data are sufficient to assess the reproductive toxicity of this material.

Developmental Toxicity

Adequate developmental toxicity studies of diglyme have been conducted using rats, mice and rabbits. Diglyme has consistently produced results that indicate it is a specific developmental toxin in experimental animals. A total of five definitive or screening studies were located in the open literature and these are shown in Table 5 with the maternal and fetal NOAELs. The two mouse studies at the bottom of the table do not have entries for NOAELs because, with few dose levels and limited fetal examinations, they were not designed to identify a NOAEL but were designed simply to indicate the potential for hazard.

Species	Route	Notes	Dev Tox ?	**Maternal NOAEL**	**Fetal NOAEL**	**Reference**
rats	Inhalation	Used 2-methoxyethanol as a positive control	+	25 ppm (100 ppm)*	<25 ppm (25 ppm)*	DuPont 1988 (55); Driscoll et al. 1998 (56)
rabbits	gavage	Reassessed NOAEL in journal publication	+	25 mg/kg**	50 mg/kg**	NTP 1987 (57); Schwetz et al. 1992 (58)
mice	gavage	**Four-dose study**	**+**	500 mg/kg	62.5 mg/kg	NTP, 1985 (59): Price et al. 1987 (60)
mice	gavage	Limited fetal examination, Positive result	+			Hardin & Eisenmann,1987 (61)
mice	gavage	Chernoff-Kavlock test, Positive result	+			Schuler et al. 1984 (62)

* = Alternative interpretation of NOAEL

** = These NOAELs are controversial (see text below)

Table 5. Available Developmental Toxicity Studies of Diglyme

These positive finding in experimental animals are not surprising in light of the information about metabolism of a fraction of diglyme to 2-methoxyacetic acid in animal models. 2-Methoxyacetic acid has been implicated as the proximate developmental toxin of 2-methoxyethanol and its occurrence as a significant metabolite of diglyme links 2-methoxyethanol and diglyme through a common metabolite. It must be keep in mind, however, that 2-methoxyacetic acid is not a necessary metabolite of diglyme and its existence and relative quantity could vary across species and among individuals depending on genetic makeup and environmental conditions that affect enzyme expression. In addition to these metabolic aspects, risk to humans is dependent on exposure, other

pharmacokinetic determinants and pharmacodynamics factors; most of which have not been fully characterized. Further discussion of human risk assessment, however, is beyond the scope of this document.

The DuPont inhalation study used dose levels of 0, 25, 100 or 400 ppm diglyme as a vapor to expose pregnant rats using a "nose only" procedure where the rats were restrained in stainless steel cylinder during the six hours of exposure. The study also used a positive control substance, 2-methoxyethanol, at a concentration of 25 ppm (equimolar basis with 25 ppm diglyme but about half the amount on a weight basis). At an exposure concentration of 400 ppm, all litters were completely resorbed and maternal toxicity was manifest as reduced food consumption. Animals exposed to 100 ppm had increased liver weights as the manifestation of maternal toxicity. Malformations were found in low incidences at 25 ppm and 100 ppm and included abnormally formed tails, distended lateral ventricles of the brain, axial skeletal malformations (vertebral fusions, hemivertebrae), and appendicular malformations (aberrant clavicular and scapular formation, bent fibula, radius, tibia, and ulna). Structural variations, primarily delayed ossification, were found in both these groups. The lowest dose of 25 ppm (140 mg/m3) caused a slightly increased incidence of variations. Although these defects were not significantly different from control values (except for the incidence of skeletal developmental variations), the pattern, type, and incidence of variations were similar to those seen at the effect level of 100 ppm. The authors, based on this similarity, suggested that 25 ppm was an effect level that approaches the lower end of the developmental toxicity response curve. Therefore, 25 ppm was considered a LOAEL for developmental effects and a NOAEL for maternal effects.

The positive control group exposed to 25 ppm 2-methoxyethanol manifest maternal toxicity as decreased feed consumption and increased liver weights. It was noted by the authors that the incidence and severity in the diglyme and 2-methoxyethanol groups exposed to 25-ppm vapors was essentially the same suggesting similar potency for producing structural variations. This comparison, however, is confounded by the maternal toxicity reported for the 2-methoxyethanol exposed dams and by the unknown contribution of the added maternal stress of being tightly confined for 6-hours a day during the nose-only exposure. Another confounding factor in these conclusions is that fetuses from the 25-ppm diglyme exposed group were not statistically affected except for a slight increase in variations over the control group.

In light of the low exposure level calculated on a mg/kg basis for the putative 25 ppm LOAEL and the confounding factors listed above, a possible reinterpretation of the results to view the maternal liver weight gain as an adaptive change and not a manifestation of toxicity, accompanied by accepting the statistical interpretation that the 25 ppm diglyme exposed fetuses are not different from controls might be a reasonable alternative interpretation. In this case the maternal NOAEL becomes 100 ppm and the developmental NOAEL would be set at 25 ppm. This approach allows a definitive NOAEL, which is better for risk assessment purposes and brings the data more in line with data from other glycol ethers in the literature and the positive control findings. An additional factor that must be taken into consideration when comparing diglyme with 2-methoxyethanol is that on a ppm basis there is gravimetrically twice the dose of diglyme being delivered as 2-methoxyethanol and as pointed out in the metabolism section, one diglyme molecule has the potential to be converted into two 2-methoxyacetic acid molecules.

The rabbit gavage study was conducted by NTP using timed pregnant New Zealand White rabbits (15-22 dams per group) dosed by water gavage on gestational days (gd) 6 through 19 at doses of 0, 25, 50, 100 or 175 mg/kg-day (57, 58). Doses were selected based on the results of preliminary dose range-finding studies. Treated females were sacrificed on gestational day 30, uterine contents were inspected, and live fetuses examined for malformations.

Clear evidence of maternal toxicity was only observed at the 175 mg/kg-day dose level where mortality among treated females was 15.4% as compared to 4% among controls. Maternal toxicity may have occurred in all groups of diglyme treated animals as body weight gain during the treatment period in these groups was less than in controls; however, corrected body weigh gain of dams did not differ between groups. Because of this, the maternal NOALE is controversial (*vide post*).

At 50 mg/kg-day, apparent adverse effects on prenatal growth, viability and morphological development were in accord with significant dose-response relationships observed across all groups, bur no individual measure reached statistical significance. At dose levels of 100 or 175 mg/kg-day, adverse effects upon fetal weight were in agreement with significant dose-response relationships, but individual treatment groups did not differ significantly from controls. The incidence of resorptions and malformed live fetuses, as well as other cumulative indices which included these endpoints, were significantly above control incidence only at dose levels of 100 and 175 mg/kg-day. Major malformations included development of the digits, craniofacial structures, abdominal wall, cardiovascular system, urogenital organs and axial skeleton. The most frequently observed individual defects were fusion of ribs (19%), hydronephrosis (23%), and clubbing of the limbs (19%) without underlying bone deformities. The incidence of adverse developmental effects was increased at a dose associated with increased maternal mortality (175 mg/kg-day). The principal manifestations of developmental toxicity at this dose were increased resorptions and a higher incidence of major malformations among surviving fetuses.

The originally published NTP report of this study published in 1987 offered the 100-mg/kg dose level as the maternal NOAEL and the 25 mg/kg level as the developmental NOAEL (57). When published in a peer-reviewed journal in 1992, however, the data had been reinterpreted and the maternal NOAEL was restated as 25 mg/kg and the developmental NOAEL as 50 mg/kg (58). This reinterpretation changes the Adult/Developmental ratio (A/D ratio), a common metric in developmental toxicity hazard assessment, from a value of 4.0 to a value of 0.5; this is a change that typically represents a reversal in developmental hazard assessment. Furthermore, in a review of the diethylene and triethylene glycol ethers' reproductive and developmental effects in 1996, Kimmel states that the maternal and developmental NOAEL for diglyme in this rabbit study are both 25 mg/kg-day (63), referencing the 1992 publication (58) as the source of this information. These discrepancies are an example of the difficulty of determining a robust maternal NOAEL in the face of actual developmental toxicity affecting total body weight gain of the pregnant dams. In spite of these limitations, the study does provide information that the rabbit is a sensitive species and that the malformations reported did not affect an individual organ system but were more nonspecific and primarily associated with visceral and external malformations and not the skeletal system.

The two screening studies listed in the table provide additional supporting evidence that diglyme has potential to be a developmental toxin in mice of other strains (61, 62). Taken together, all five of these studies provide consistent and convincing evidence that diglyme is a developmental toxin in experimental animals. Additional studies on the metabolism of diglyme to 2-methoxyacetic acid in experimental animals strengthen this conclusion and provide a mechanistic basis for the developmental toxicity of diglyme.

Recommendation: No additional developmental toxicity testing is required as the available data are sufficient to assess the developmental toxicity of this material relative to the requirements of the HPV program.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, it is concluded that the available information fills all of the requirements for physicochemical parameters, fate information, aquatic toxicity and mammalian toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, taken together the information provides a reliable hazard assessment. Conduct of additional SIDS-screening studies would not add significantly to our understanding of this material's toxicity.

References

1 Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 12th ed. New York, NY: Van Nostrand Rheinhold Co., 1993 page 392

2 O'Neil, MJ (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Thirteenth edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001

3 Stoye, D. *Solvents* in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VHC article dated 15 June 2000.

4 NIOSH/CDC. NIOSH Recommendations for Occupational Safety and Health Standards 1988, Aug. 1988. (Suppl. to *Morbidity and Mortality Wkly.* Vol. 37 No. 5-7, Aug. 26, 1988). Atlanta, GA: National Institute for Occupational Safety and Health, CDC, 1988

5 Chemical Information System, NISC International, Inc. Baltimore, Maryland

6 Daubert, T.E., R.P. Danner. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis, 1989 as cited in National Library of Medicine Hazardous Substance Data Base, Record #72, Last Revision Date: 20030305

7 Hansch, C., Leo, A., D. Hoekman. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society. 1995. page 97

8 Tessier, S et al. Degradation of Polyoxyethylenes: Biodegradation using an Enzyme from Psuedomonas P-400 and Chemical Degradation using Sodium Hypochlorite. *J. Chem research* 1983: 174-175 (1983)

9 Roy, D. Anagnostu, G. Chaphalkar, P. Biodegradation of Dioxane and Diglyme in Industrial Waste. *Journal of Environmental Science and Health Part A Environmental Science And Engineering*; 29 (1). 1994. 129-147

10 Cowan, RM, et al. Activated Sludge and Other Aerobic Suspended Culture Processes Water Environment Research; 67 (4). 1995. 433-450

11 International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

12 European Chemicals Bureau, IUCLID 2000 for CASNO 111-96-6

13 Hoechst AG (1989): Unveroeffentlichte Untersuchung (V–89–903–A) as cited in European Chemicals Bureau, IUCLID 2000 for CASNO 111-96-6

14 Hoechst (1989) *Prüfberichte: ökologische Untersuchungen.* Frankfurt am Main, Hoechst AG, 4 pp. (unpublished report). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

15 Grossmann D, Koser H, Kretschmer R, Porobin M. Treatment of diglyme containing wastewater by advanced oxidation--process design and optimisation. *Water Sci Technol*. 44:287-93 (2001).

16 Dagaut P, Wallington IJ, Liu R, Kurylo MJ (1988) 22nd International Symposium on Combustion, Seattle, WA; as cited in Atkinson R (1989) Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds. Journal of Physical and Chemical Reference Data, 1:143. as cited in Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

17 Teratologic evaluation of diethylene glycol dimethyl ether (CAS No. 111-96-6) administered to New Zealand White rabbits on gestation days 6 through 19. (1987) Research Triangle Park, NC, National Institute of

Environmental Health Sciences, National Toxicology Program (NTP-87-108; PB 87-209532). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

18 J.C. Harris in Lyman W, Reehl, W and Rosenblat, D.(1990) Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington D.C.

19 See http://www.glymes.com/diglyme.php for examples.

20 EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

21 Hoechst Abwasserbiologische Untersuchung von Dialkylglykoläthern auf die Goldorfe (Leuciscus idus). Report Nr.79.0555 (1979) as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002 and using data found in the ECB IUCLID 2000 document for diglyme.

22 Hoechst (1994) Prüfung der toxischen Wirkung von Diethylenglykoldimethylether auf Kleinkrebse (Daphnia magna). Hoechst AG, Frankfurt am Main (unpublished report) .as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

23 Hoechst (1995) Prüfung der Schadwirkung gegenüber Algen (Algentoxizität) von Diethylenglykoldimethylether. Hoechst AG, Frankfurt am Main (unpublished report). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

24 ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

25 Daniel FB, Cheever KL, Begley KB, Richards DE, Weigel WW, Eisenmann CJ Bis(2-methoxyethyl) ether: metabolism and embryonic disposition of a developmental toxicant in the pregnant CD-1 mouse. Fundam Appl Toxicol 16:567-75 (1991).

26 Tirmenstein MA Comparative metabolism of bis(2-methoxyethyl) ether by rat and human hepatic microsomes:Formation of 2-methoxyethanol. *Toxicology in vitro* ,7:645–652 (1993)

27 Toraason M, Richards DE, Tirmenstein MA (1996) Metabolism of diglyme by rat hepatocytes and human microsomes. *Occupational hygiene*, 2:33–43 (1996).

28 Cheever KL, Richards DE, Weigel WW, Begley KB The role of enzyme induction on metabolite formation of bis(2-methoxyethyl) ether in the rat. *Toxicology and industrial health* 5:601–607 (1989).

29 Cheever KL, Richards DE, Weigel WW, Lal JB, Dinsmore AM, Daniel FB (1988) Metabolism of bis(2-methoxyethyl) ether in the adult male rat: evaluation of the principal metabolite as a testicular toxicant. *Toxicology and applied pharmacology*, 94:150–159

30 The Toxicology of Glycol Ethers and its Relevance to Man. Tech Report No. 64 ECETOC (European Centre for Ecotoxicology and Toxicology of Chemical) Brussels, Belgium 1995.

31 DuPont Chemical, Haskell Laboratories report 51-73. Initial Submission: Acute Oral Test of Ether,Bis(2-Methoxyethyl) In Rats With Cover Letter Dated 101592. U.S. EPA/OPTS Public Files, Document Number: 88-920009811, TSCATS mfiche.

32 Hoechst AG Akute orale Toxizität von Diethylenglykoldimethylether an weiblichen Ratten. Frankfurt am Main, Hoechst AG, 4 pp. (Report 376/79; unpublished) (1979). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002 and data as found in ECB IUCLID 2000 document for diglyme.

33 Plasterer MR, Bradshaw WS, Booth GM, Carter MW, Schuler RL, Hardin BD. Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: naphthalene, p-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea, and four glycol ether derivatives. Journal of Toxicology and Environmental Health, 15:25–38 (1985).

34 Hoechst (1979a) *Inhalationstoxizität im Zeitsättigungstest von Diethylenglykol-dimethylether an männlichen und weiblichen SPF-Wistar-Ratten.* Frankfurt am Main, Hoechst AG, 10 pp . (Report 488/79; unpublished) as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

35 Hoechst AG : Unveroeffentlichte Untersuchung (79.0488) 1979 as cited in ECB IUCLID-2000 for diglyme.

36 McGregor DB, Willins MJ, McDonald D, Holmstrom M, McDonald D, Niemeier RW Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl)ether. *Toxicology and Applied Pharmacology*, 70:303–316 (1983).

37 Valentine R, O'Neill AJ, Lee KP, Kennedy GL Jr. Subchronic inhalation toxicity of diglyme. *Food Chem Toxicol*. 37:75-86 (1999).

38 Larese Filon F, Fiorito A, Adami G, Barbieri P, Coceani N, Bussani R, Reisenhofer E. Skin absorption in vitro of glycol ethers. *Int Arch Occup Environ Health.* 72:480-4 (1999).

39 Dugard PH, Walker M, Mawdsley SJ, Scott RC. Absorption of some glycol ethers through human skin in vitro. *Environ Health Perspect.* 57:193-7 (1984)

40 Carpenter, CP et al. The Toxicity of Butyl Cellosolve Solvent. *AMA Arch Ind Health* 14:114 (1956).

41 Carpenter, CP Queries and Minor Notes. *J Am Med Assoc*. 135:880 (1947).

42 Symth, HF. Seaton, J and L. Fisher The Single Dose Toxicity of Some Glycols and Derivatives. *J Ind Hyg Toxicol*. 23:259-268 (1941).

43 OECD Dermal Acute Toxicity Guideline 402 (24 February 1987).

44 Nagano K, Nakayama E, Oobayashi H, Nishizawa T, Okuda H, Yamazaki K. Experimental Studies on Toxicity of Ethylene Glycol Alkyl Ethers in Japan. *Environmental Health Perspectives*, 57:75–84 (1984).

45 Valentine R, O'Neill AJ, Lee KP, Kennedy GL Jr. Subchronic inhalation toxicity of diglyme. *Food Chem Toxicol.* 37:75-86 (1999)

46 DuPont (1989) *Subchronic inhalation toxicity study with diglyme.* Newark, NJ, E.I. Du Pont de Nemours & Co., 187 pp . as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

47 Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E (1986) *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environmental mutagenesis*, 8(S7):1–119 .

48 Data from this study is found on the NTP public database at http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm

49 McGregor DB, Willins MJ, McDonald D, Holmstrom M, McDonald D, Niemeier RW (1983) Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl)ether. Toxicology and Applied Pharmacology, 70:303–316.

50 Hoechst (1979) *Test for mutagenicity in bacteria strains in the absence and presence of a liver preparation*. Frankfurt am Main, Hoechst AG, 7 pp. (Report 53/79; unpublished). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

51 Hoechst (1979) *Mutagenicity evaluation of diethyleneglycoldimethylether in the Ames* Salmonella*/microsome plate test.* Frankfurt am Main, Hoechst AG, 15 pp. (Report 743/79, unpublished). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

52 ECETOC *The toxicology of glycol ethers and its relevance to man.* Brussels, European Centre for Ecotoxicology and Toxicology of Chemicals, pp. 1–350 (Technical Report No . 64), 1995.

53 Cheever KL, et al. Testicular Effects of Bis(2-methoxyethyl) Ether in the Adult Male Rat Toxicology and Industrial Health, 5:1099-1109 (1989)

54 McGregor DB. Genotoxicity of Glycol Ethers Environmental Health Perspectives 57:97-103 (1984).

55 DuPont Haskell Laboratory *Teratogenicity study of diglyme in the rat* . Newark, NJ, E.I. Du Pont de Nemours & Co., 289 pp (1988).

56 Driscoll CD, Valentine R, Staples RE, Chromey NC, Kennedy GL Jr Developmental toxicity of diglyme by inhalation in the rat. *Drug and chemical toxicology*, 21(2):119–136 (1998).

57 NTP Teratologic evaluation of diethylene glycol dimethyl ether (CAS No. 111-96-6) administered to New Zealand White rabbits on gestation days 6 through 19. Research Triangle Park, NC, National Institute of Environmental Health Sciences, National Toxicology Program NTP-87-108; PB 87-209532, (1987)

58 Schwetz BA, Price CJ, George JD, Kimmel CA, Morrissey RE, Marr MC The developmental toxicity of diethylene and triethylene glycol dimethyl ethers in rabbits. *Fundamental and applied toxicology*, 19(2):238–245 (1992).

59 Teratologic Evaluation of Diethylene Glycol Dimethyl Ether (CAS NO. 111-96-6) Administered to CD-1® Mice on Gestational Days 6 through 15. NTP Study TER85061, Report Date: August 30, 1985 NTIS#PB86-135233

60 Price CJ, Kimmel CA, George JD, Marr MC. The developmental toxicity of diethylene glycol dimethyl ether in mice. *Fundamental and applied toxicology*, 8:115–126 (1987).

61 Hardin BD, Eisenmann CJ (1987) Relative potency of four ethylene glycol ethers for induction of paw malformations in the CD-1 mouse. *Teratology*, 35:321–328

62 Schuler RL, Hardin BD, Niemeier RW, Booth G, Hazelden K, Piccirillo V, Smith K (1984) Results of testing fifteen glycol ethers in a short-term *in vivo* reproductive toxicity assay . *Environmental health perspectives*, 57:141–146 .

63 Kimmel CA. Reproductive and developmental effects of diethylene and triethylene glycol (methyl-, ethyl-) ethers. *Occupational hygiene*, 2:131–151(1996)